A TAXONOMIC STUDY OF THE GENUS *LAURENCIA* (CERAMIALES, RHODOPHYTA) FROM VIETNAM. V. *LAURENCIA CONCRETA* CRIBB AND *L. DINHII* SP. NOV.

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ABSTRACT - - The red alga *Laurencia concreta* Cribb (Rhodomelaceae, Ceramiales), reported for the first time from Vietnam, is characterised by the production of two vegetative periaxial cells from each axial segment. This, in combination with other features, confirms its inclusion in the subgenus *Chondrophycus*. *Laurencia dinhii* Masuda *et* Kogame, sp. nov., endemic to southern Vietnam, is characterised by the following set of morphological features: 1) a basal system composed of a single disc from which a few terete to subterete axes arise; 2) the production of two vegetative periaxial cells from each axial segment; 3) the presence of a palisade-like superficial cortical layer; 4) the absence of projecting superficial cortical cells; 5) the absence of longitudinally oriented secondary pit-connections between contiguous superficial cortical cells; 6) the absence of lenticular thickenings in the walls of medullary cells; 7) the presence of intercellular spaces between contiguous medullary cells; 8) a perpendicular arrangement of tetrasporangia, each of which is produced from the second periaxial cell in each fertile segment; 9) procarps produced from the last-formed (fourth) periaxial cell of the terminal segment of a two-celled female trichoblast; and 10) flask-shaped cystocarps.

RÉSUMÉ - L'algue rouge *Laurencia concreta* Cribb (Rhodomelaceae, Ceramiales), signalée ici pour la première fois au Vietnam, est caractérisée par la production de deux cellules périaxiales végétatives à partir de chaque segment axial. Combiné à d'autres caractéristiques, ceci confirme son inclusion dans le sous-genre *Chondrophycus*. *Laurencia dinhii* Masuda *et* Kogame, sp. nov., endémique du Sud-Vietnam, est définie par l'ensemble des caractéristiques morphologiques suivantes : 1) un système basal composé d'un disque unique à partir duquel sont émis des axes à section circulaire ou sub-circulaire ; 2) la production de deux cellules périaxiales à partir de chaque segment axial ; 3) la présence d'une assise de cellules corticales superficielle palissadique ; 4) l'absence de cellules corticales superficielles saillantes ; 5) l'absence de synapses secondaires orientées longitudinalement entre les cellules corticales superficielles ; 6) l'absence d'épaississements lenticulaires dans les parois des cellules médullaires ; 7) la présence d'espaces intercellulaires entre les cellules médullaires contiguës ; 8) une disposition des tétrasporocystes perpendiculaire à l'axe longitudinal, chacun des tétrasporocystes étant produit à partir de la seconde cellule périaxiale de chaque segment fertile ; 9) des procarpes produits à partir de la dernière (quatrième) cellule périaxiale formée par le segment terminal d'un trichoblaste femelle bicellulaire ; et 10) des cystocarpes en forme d'ampoule. (Traduit par la Rédaction)

KEY WORDS: Ceramiales, *Laurencia concreta*, *Laurencia dinhii*, Rhodomelaceae, Rhodophyta, Taxonomy, Vietnam.

INTRODUCTION

The red algal genus *Laurencia* (Rhodomelaceae, Ceramiales) includes two subgenera, *Laurencia* and *Chondrophycus* Tokida *et* Saito (Saito, 1967). These subgenera were originally separated on the basis of the presence or absence of longitudinally oriented secondary pit-connections between contiguous superficial cortical cells and the tetrasporangial arrangement, whether parallel or perpendicular (Saito, 1967). However, more recent studies of the genus have shown that these criteria do not allow the unequivocal subgenus placement of all species (Masuda *et al.*, 1998 and references cited therein), and that the number of vegetative periaxial cells produced from each vegetative axial segment is the most consistent feature distinguishing members of *Laurencia* from those of *Chondrophycus*: four in the former and two in the latter (Nam & Saito, 1995). Many species assignable to *Chondrophycus* in the sense of Saito (1967) need to have their number of periaxial cells documented in order to be confirmed as members of that subgenus. In this paper, one such species, *L. concreta* Cribb, and a new species, *L. dinhii*, are reported from Vietnam.

MATERIALS AND METHODS

Specimens of *Laurencia concreta* were collected at Tam Quan (11.iii.1994, *leg.* T. Abe), Nui Thanh, Quang Nam-Da Nang Province, Hon Tre Island (6.iii.1992, *leg.* M. Masuda), Hon Rua (23.i.1993, *leg.* M. Masuda), Nha Trang, Khanh Hoa Province, My Hoa (20.i.1993, *leg.* M. Masuda), Ninh Hai, Ninh Thuan Province, and An Thoi (8.ii.1993, *leg.* M. Masuda), Phu Quoc Island, Kien Giang Province; plants of *L. dinhii* were collected at My Tuong (7.iii.1992, *leg.* M. Masuda), Phan Rang, and Son Hai (21.i.1993, *leg.* M. Masuda), Ninh Phuoc, Ninh Thuan Province. Material was fixed in 10% formalin in seawater, with some being pressed as voucher herbarium specimens. Sections were made by hand using a razor blade and pith stick. They were mounted in 50% glycerol-seawater on microscope slides and stained with 0.5% (w/v) cotton blue in a lactic acid/phenol/glycerol/water (1:1:1:1) solution. Specimens are deposited in the Herbarium of the Graduate School of Science, Hokkaido University (SAP 062601-062610).

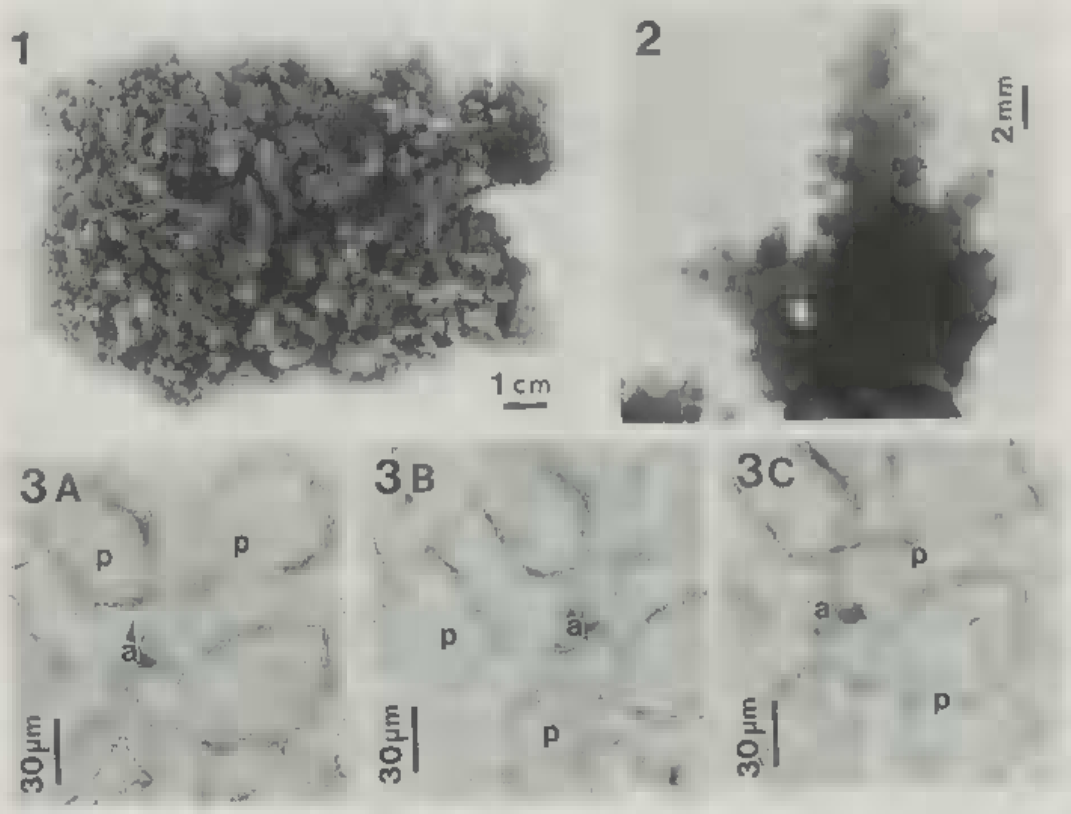
OBSERVATIONS

***Laurencia concreta* Cribb 1983: 116**

Type locality. Fairfax Island, the Great Barrier Reef, Australia; holotype specimen in the Herbarium of Department of Botany, University of Queensland, Saint Lucia, Australia (BRIU 877.18).

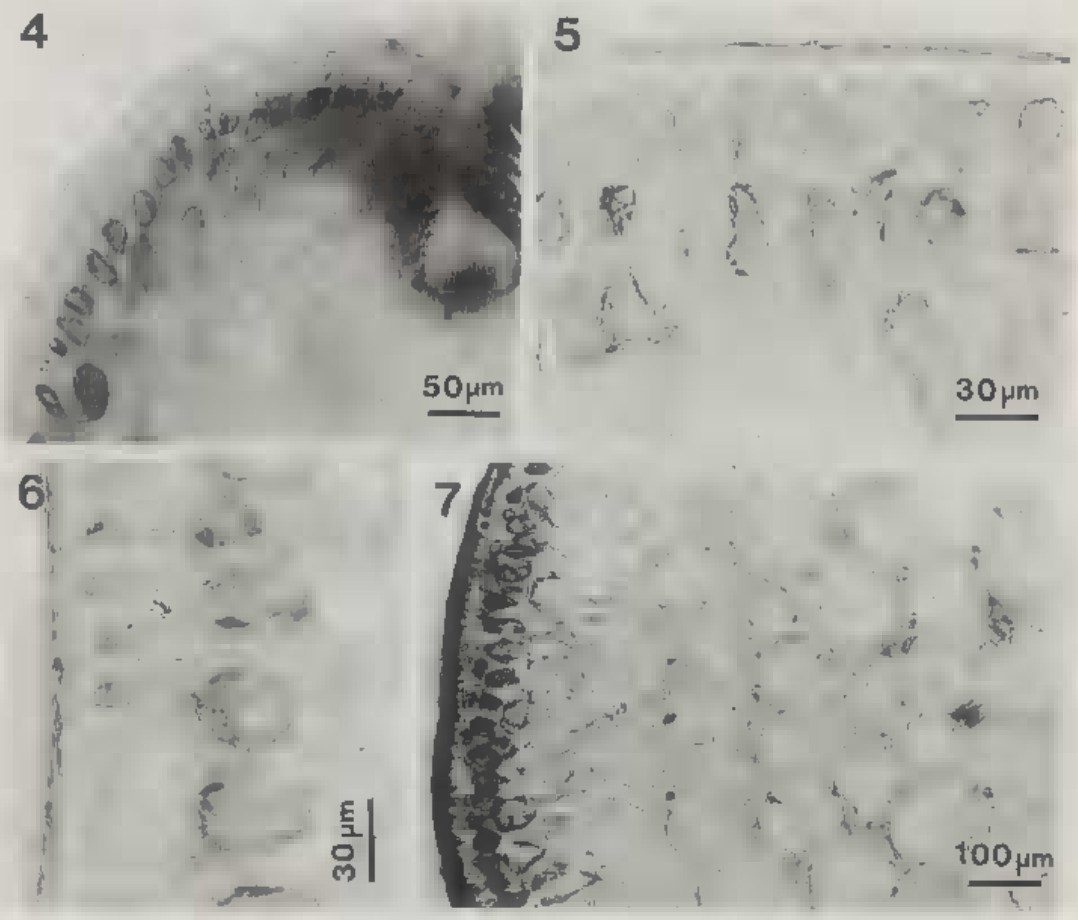
Distribution. Tropical and subtropical regions in the southwestern and western Pacific: eastern Australia (Cribb, 1983), Borneo (Masuda, unpublished observations), Vietnam (Dawson, 1954, as *L. paniculata* [C. Agardh] J. Agardh; present study) and the Ryukyu Islands, Japan (Masuda, unpublished observations).

Plants grow on rocks in the lower intertidal to upper subtidal zones on open and sheltered reef flats, where they form irregular or circular, cushion-shaped clumps (Fig. 1) up to 20 cm in diameter attached at many points to substrata by primary and secondary holdfasts. Thalli are brownish-purple and rigidly cartilaginous, but are crisp when living. Each clump is composed of densely entangled, terete to subterete (frequently angular at branch points) axes and branches which are free at the distal portions. Closely-placed branches are usually linked by anastomoses and cannot be separated without fragmenting the thallus. Axes are branched in an irregularly spiral manner (Fig. 2) at intervals of 0.5-1.5 mm and at angles of 40-90°, but percurrent main axes are not discernible. Large branches are 2.5-3.2 mm in diameter at middle portions and 0.9-1.5 mm distally. Branches of all orders are polystichous (arranged in many rows). The majority of penultimate branches are short, 1.0-2.5 mm long and 0.9-1.5 mm in diameter and bear a few ultimate branches that are 0.5-1.0 mm long and 0.4-1.0 mm in diameter (Fig. 2).



Figs 1-3. *Laurencia concreta* Cribb. My Hoa, Ninh Hai, Ninh Thuan Province, Vietnam. Fig. 1. Formalin/seawater-preserved specimen (SAP 062602). Fig. 2. Distal portion of a branch. Figs 3A-C. Transverse section (TS) of the upper portion of a penultimate branch (at three different focal planes), showing each axial cell (a) connected with two periaxial cells (p) that arise approximately 120° angles to one another.

The growing point is always immersed in an apical pit, as is typical of the genus. Each axial cell produces two periaxial cells (Figs 3A-C). Periaxial cells cut off from three, successive axial cells are arranged at 120° angles to one another (Figs 3A-C), and the periaxial-cell pairs are rotated in a 1/3 spiral. This arrangement and successive, radial production of cells from each periaxial cell form the terete thallus. Superficial cortical cells are polygonal to elliptical in surface view and regularly arranged in longitudinal rows. They are 8-20 μm long by 10-24 μm wide (a length:width ratio of 0.5-0.9) in surface view in the distal portions of large branches, 14-40 μm long by 10-26 μm wide (a length:width ratio of 1.0-2.5) in the middle portions, and 12-28 μm long by 16-48 μm wide (a length:width ratio of 0.4-1.0) in the proximal portions. Superficial cortical cells do not project at the apices of ultimate branchlets (Fig. 4).



Figs 4-7. *Laurencia concreta* Cribb. My Hoa, Ninh Hai, Ninh Thuan Province, Vietnam. Fig. 4. Longitudinal section (LS) of the uppermost portion of an ultimate branch. Fig. 5. TS of the upper portion of a penultimate branch, showing a palisade-like superficial cortical layer. Fig. 6. LS of the middle portion of a branch, showing the absence of longitudinally oriented secondary pit-connections between contiguous superficial cortical cells. Fig. 7. TS of the lower portion of a branch, showing the absence of intercellular spaces.

Superficial cortical cells are radially elongated, form a continuous palisade-like layer (Figs 4-6) and in transverse sections are 24-36 μm thick (a thickness:width ratio of 1.5-2.3) in the distal portions of large branches and 24-50 μm thick (a thickness:width ratio of 1.2-2.9) in the middle to proximal portions. Longitudinally oriented secondary pit-connections are absent between contiguous superficial cortical cells (Fig. 6). Lenticular thickenings are also absent in the walls of medullary cells (Fig. 7), which are 80-240 μm in diameter, and have walls of 4-10 μm in thickness in the middle to proximal portions of large branches. Cortical and medullary cells are closely packed, and intercellular spaces are absent between contiguous cells (Fig. 7). Reproductive plants were not observed.

***Laurencia dinhii* sp. nov.**

Plantae singulae ex axibus rectis aliquot e disco basali communi effecti constantes, destitutae ramos repentes infernos adhaesionibus secundariis; thalli recti fertiles 6-11 cm alti, teres ad subteres omnino, axibus principalibus percurrentibus, luteo-ad viridi-purpurei, cartilaginei, elastici, exsiccatione chartae adhaerentes; axes principales usque ad 1.8-2.3 mm diametro, ramos in modo spirali ferentes; cellula axialis omnis cum cellulis 2 periaxialibus; foveae-colligationes secundariae longitudinaliter dispositae inter cellulas corticales superficiales contingentes absentes; cellulae corticales superficiales in sectionibus transversalibus ramuli radiatim elongatae et in strato valliformi continuo dispositae; incrassationes lenticulares in parietibus cellularum medullae absentes; spatia intercellulosa inter cellulas medullosas contiguas adsunt; tetrasporangia in ramis ultimis et penultimis in ordinatione perpendiculari ad axem longitudinalem formata, unumquidque e cellula periaxiali secunda segmenti fertilis factum; tetrasporangia matura 140-160 μm longa et 80-100 μm diametro; segmentum procarpiumferum cellulas 4 periaxialem efferens; cystocarpia laterales in ramis, lageniformia, 720-960 μm alta et 700-920 μm diametro, collo elevato 120-360 μm alto; spermatangia ignota.

Individual plants consisting of several upright axes arising from a common discoid holdfast, lacking lower creeping branches with secondary attachments; fertile thalli 6-11 cm high, terete to subterete throughout, with percurrent main axes, yellowish- to greenish-purple, cartilaginous, elastic, adhering to paper on drying; main axes up to 1.8-2.3 mm in diameter, bearing branches in a spiral manner; each axial cell with 2 periaxial cells; longitudinally oriented secondary pit-connections absent between contiguous superficial cortical cells; superficial cortical cells in transverse sections of branchlets elongated radially and arranged in a continuous palisade-like layer; lenticular thickenings absent in the walls of medullary cells; intercellular spaces present between contiguous medullary cells; tetrasporangia formed in ultimate and penultimate branches in a perpendicular arrangement to the longitudinal axis, each produced from the second periaxial cell of fertile segment; mature tetrasporangia 140-160 μm long and 80-100 μm in diameter; procarp-bearing segment producing four periaxial cells; cystocarps lateral on branches, flask-shaped, 720-960 μm high and 700-920 μm in diameter, with elevated neck 120-360 μm high; spermatangia unknown.

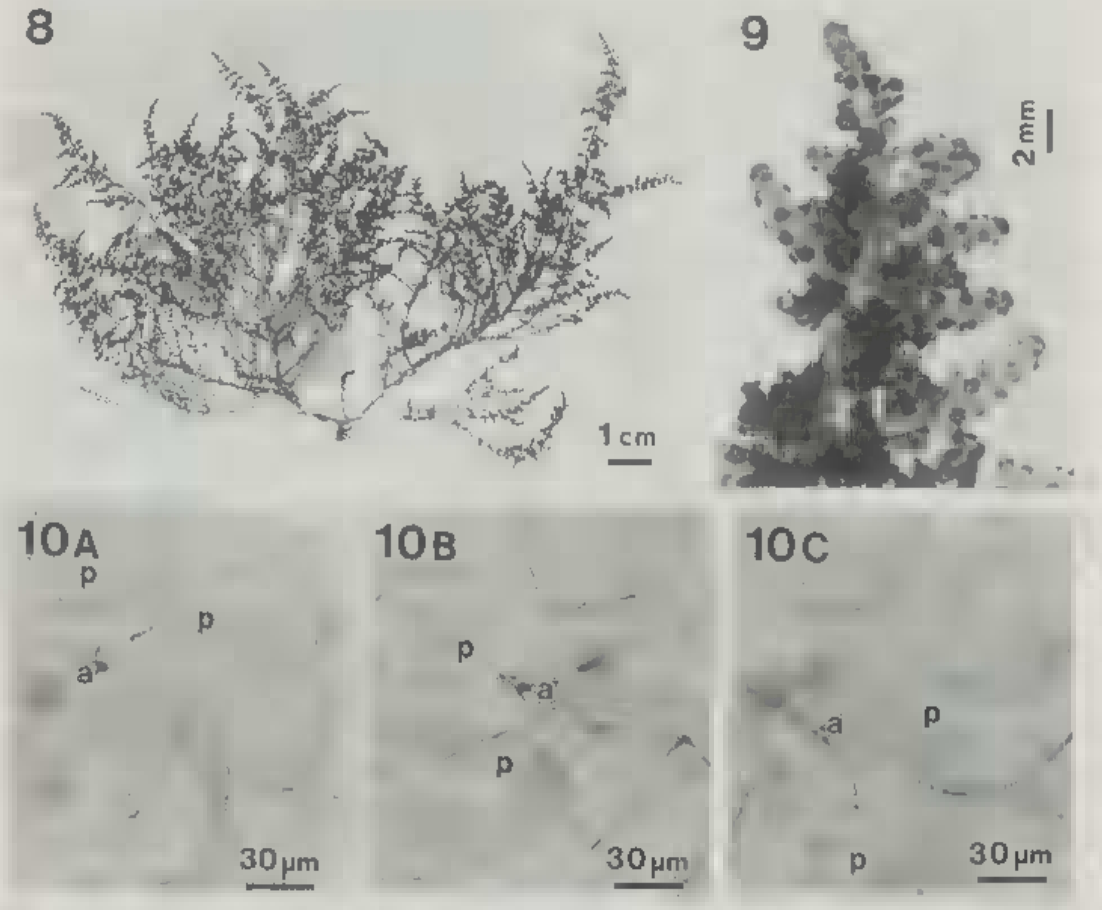
Holotype and type locality. Cystocarpic plant deposited in SAP (062606) (Fig. 8), collected by M. Masuda on 7 March 1992 at My Tuong, Phan Rang, Ninh Thuan Province, Vietnam.

Distribution. Endemic to southern Vietnam; known from Ninh Thuan Province, facing the South China Sea.

Etymology. The specific epithet is dedicated to Dr Nguyen Huu Dinh, who is a Senior Research Officer of the National Center for Scientific Research of Vietnam, in recognition of his many contributions to the taxonomy of marine algae in Vietnam.

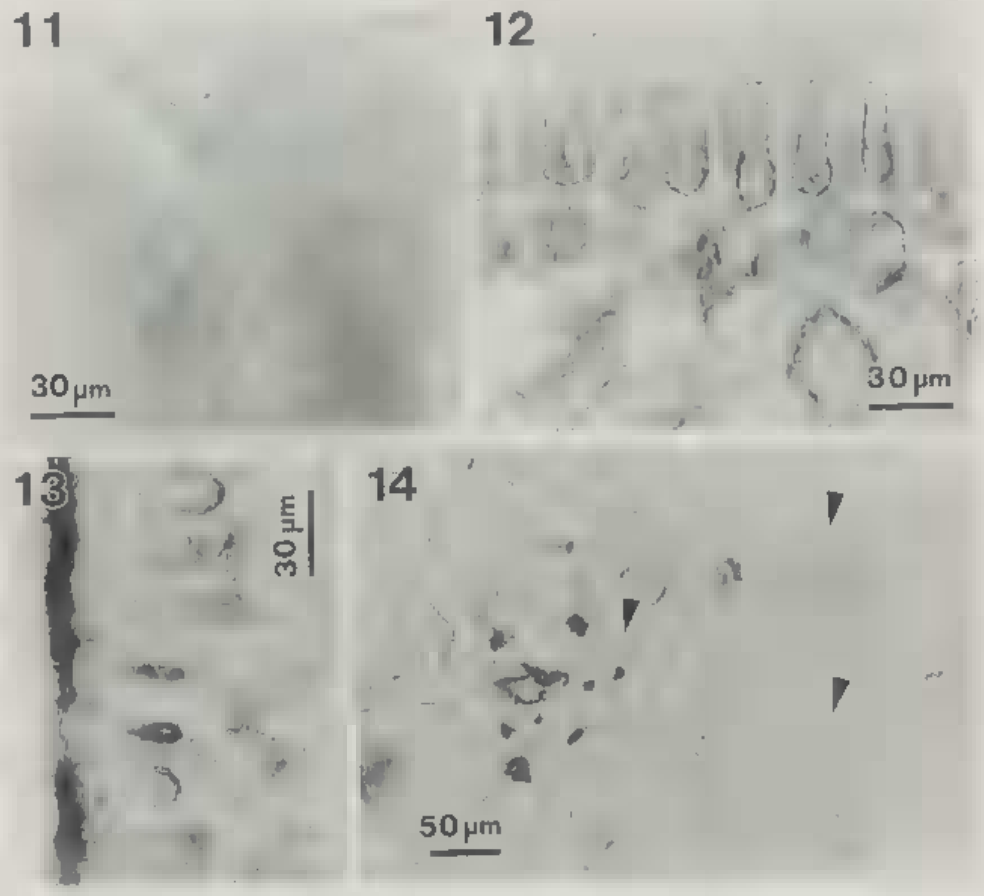
Plants grow solitarily on limestone or dead coral in the lower intertidal to upper subtidal zones on sheltered reef flats. They are 6-11 cm long (Fig. 8), yellowish-to greenish-purple, rigidly cartilaginous, terete to subterete throughout, and have percurrent main axes. One to seven upright axes arise from a common discoid holdfast 3-6 mm in diameter. Main axes are 1.2-2.0 mm in diameter in the lowest portions, 1.8-2.3 mm in the middle portions, then taper gradually upwards to 0.8-1.1 mm in the uppermost portions.

Many first-order branches are formed in an irregularly spiral manner 0.5-4.0 mm apart and at angles of 30-90°. These branches are 4-8 cm long on the lower to middle portions of the main axes, becoming shorter upwards. They bear progressively shorter branches of up to five orders which are also arranged in an irregularly spiral manner (Fig. 9). Ultimate branches are club-shaped or barrel-shaped, up to 600 µm long and 500-700 µm in diameter. Branches of all orders are polystichous (arranged in many rows). Adventitious branches are limited to the lower portions of the main axes.



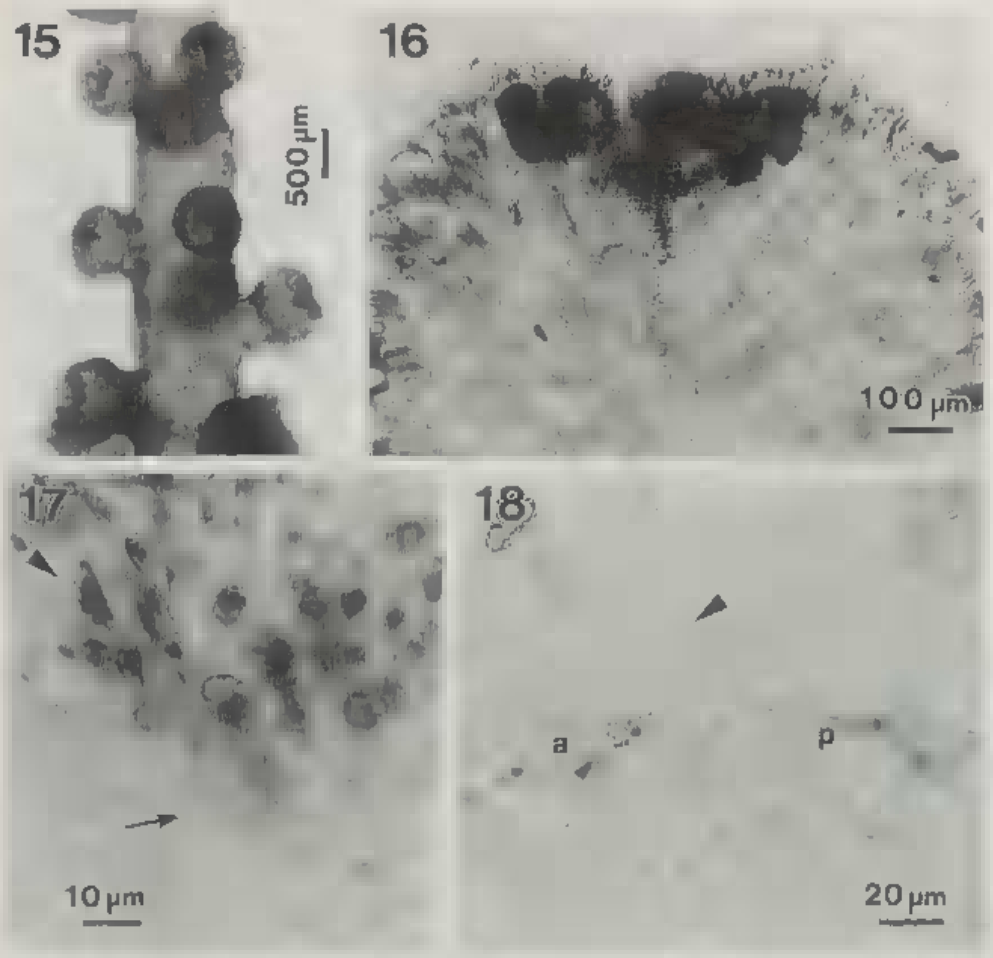
Figs 8-10. *Laurencia dinhii* Masuda *et* Kogame, sp. nov. My Tuong, Phan Rang, Ninh Thuan Province, Vietnam. Fig. 8. Holotype specimen (cystocarpic, SAP 062606). Fig. 9. Upper portion of a first-order branch. Figs 10A-C. TS of the upper portion of a third-order branch (at three different focal planes), showing each axial cell (a) connected with two periaxial cells (p) that arise approximately 120° angles to one another.

The growing point is always located within an apical pit, as is typical of the genus. Each axial cell produces two periaxial cells (Figs 10A-C). Periaxial cells cut off from three, successive axial cells are arranged at 120° angles to one another (Figs 10A-C), and the periaxial-cell pairs are rotated in a 1/3 spiral. This arrangement and successive, radial production of cells from the periaxial cells form the terete thallus. Superficial cortical cells are round, elliptical to polygonal in surface view, and regularly arranged in longitudinal rows. They are 8-16 µm long by 12-30 µm wide (a length:width ratio of 0.4-1.0) in surface views of distal portions of first-order branches, 19-90 µm long by 14-50 µm wide (a length:width ratio of 0.9-2.0) in the middle portions, and 12-40 µm long by 16-92 µm wide (a length:width ratio of 0.3-1.2) in the proximal portions. Superficial cortical cells do not project at the apices of ultimate branchlets (Fig. 11).



Figs 11-14. *Laurencia dinhii* Masuda *et* Kogame, sp. nov. My Tuong, Phan Rang, Ninh Thuan Province, Vietnam. Fig. 11. LS of the uppermost portion of a second-order branch. Fig. 12. TS of the upper portion of a third-order branch, showing a palisade-like superficial cortical layer. Fig. 13. LS of the middle portion of a first-order branch, showing the absence of longitudinally oriented secondary pit-connections between contiguous superficial cortical cells. Fig. 14. TS of the lower portion of a first-order branch, showing intercellular spaces (arrowheads) between medullary cells.

Superficial cortical cells are radially elongated, form a continuous palisade-like layer (Fig. 12), and in transverse section are 24-30 μm thick (a thickness:width ratio of 1.2-1.9) in distal portions of first-order branches, 36-100 μm thick (a thickness:width ratio of 1.3-2.4) in the middle portions, and 26-80 μm thick (a thickness:width ratio of 1.3-2.5) in the proximal portions. Longitudinally oriented secondary pit-connections are absent between contiguous superficial cortical cells (Fig. 13), and lenticular thickenings are absent in the walls of medullary cells. Medullary cells are 80-200 μm in diameter, and have walls of 6-8 μm in thickness in the middle to proximal portions of first-order branches. Intercellular spaces are present in the medullary layer (Fig. 14).



Figs 15-18. *Laurencia dinhii* Masuda *et* Kogame, sp. nov. My Tuong, Phan Rang, Ninh Thuan Province, Vietnam. Fig. 15. Upper portion of a second-order branch bearing third-order tetrasporangial branches. Fig. 16. LS of a tetrasporangial branch, showing a perpendicular arrangement of the tetrasporangia. Fig. 17. LS of a tetrasporangial branch in which a young tetrasporangium (arrowhead) is being produced abaxially on an elongated, fertile periaxial cell (arrow). Fig. 18. TS of a tetrasporangial branch, showing an axial cell (a) with an associated vegetative periaxial cell (p) and a fertile periaxial cell (arrowhead).

Tetrasporangia are formed in distal portions of ultimate and penultimate branches (Figs 15-16) which are 500-1200 μm long by 600-1000 μm wide. One (the second) of the two periaxial cells in each fertile segment substantially elongates towards the thallus surface (Figs 17-18) and produces an abaxial tetrasporangium (Fig. 17). Each tetrasporangium is provided with two cover cells that are distally produced by the fertile periaxial cell. Tetrasporangia mature centripetally and show a perpendicular arrangement relative to the longitudinal axis of the bearing branch almost until maturity (Fig. 16). Mature tetrasporangia are 140-160 μm long by 80-100 μm in diameter.

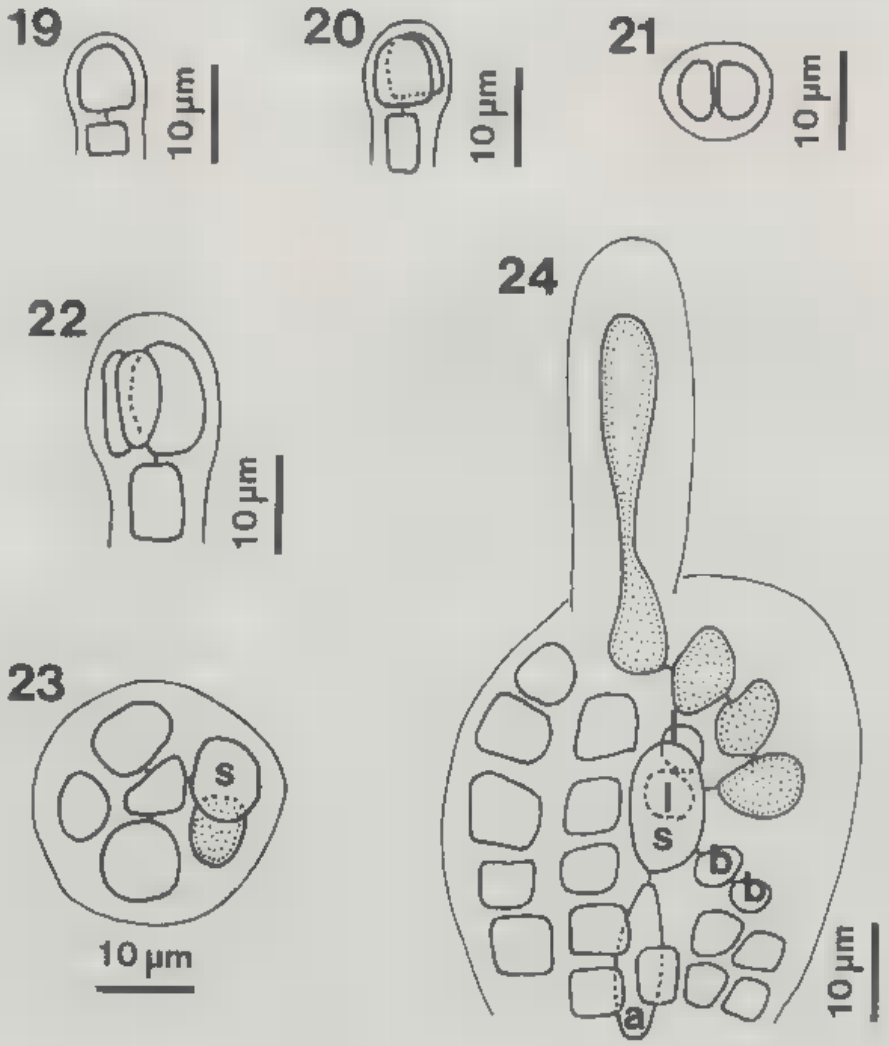
Female trichoblasts are formed in cup-shaped pits of ultimate branches. They are composed of two segments and produce a single procarp on their terminal segment (Figs 19-24). The segment bears four periaxial cells, of which the last-formed functions as the supporting cell (Fig. 23) and produces a four-celled carpogonial branch and two sterile groups, lateral (first) and basal (second) (Fig. 24). Mature procarps are covered by a pericarp and each possesses a projecting trichogyne from the apical pit. Cystocarps are lateral on branches of any order. They are flask-shaped (Figs 25, 26), 720-960 μm long and 700-920 μm wide, having extended ostiolar necks 120-360 μm in length. Spermatangial plants are unknown.

DISCUSSION

Laurencia concreta was first described by Cribb (1983) on the basis of vegetative specimens collected from Fairfax Island, Queensland, Australia. It is characterised by the following combination of features: 1) rigidly cartilaginous but crisp, interlocking, cushion-shaped thalli with attachment by holdfasts at numerous points and with closely-placed branches becoming anastomosed; 2) the presence of numerous wart-like branches; 3) the absence of longitudinally oriented secondary pit-connections between contiguous superficial cortical cells; 4) the presence of a palisade-like surface layer; 5) the absence of projecting superficial cortical cells; and 6) the absence of lenticular thickenings in the walls of medullary cells. Each axial cell, in addition, produces only two periaxial cells, a critical feature of the subgenus *Chondrophycus* (Nam & Saito, 1995).

Laurencia concreta is somewhat similar in gross morphology to *L. papillosa* (C. Agardh) Greville, but is distinguished by the production of numerous anastomosing branches that result in a tightly concrescent, cushion-shaped clump. However, it is virtually impossible to distinguish formalin/seawater-preserved, fragmentary specimens of *L. concreta* from those of *L. papillosa*.

Although this is the first confirmed record of *Laurencia concreta* outside of Australia, its occurrence in Vietnam was suggested by Cribb (1983) on the basis of illustrations given by Dawson (1954, figs 61c, d), who identified his Nha Trang specimens as *L. paniculata* (C. Agardh) J. Agardh. The present collection from Vietnam support Cribb's suggestion. Genuine *L. paniculata*, which is now known as *L. patentiramea* (Montagne) Kützing (Silva *et al.*, 1987), does not possess cushion-like thalli (Montagne, 1836, as *Chondria obtusa* C. Agardh var. *patentiramea* Montagne; Yamada, 1931, pl. 3, fig. a, as *L. paniculata*; Athanasiadis, 1987, as *L. paniculata*). According to Silva *et al.* (1996), the correct name for both *Laurencia patentiramea* and *L. paniculata* should be *Laurencia glandulifera* (Kützing) Kützing. However, Saito (1985), who examined the type material and liquid-preserved specimens of *L. glandulifera* from the Adriatic Sea, reported the



Figs 19-24. *Laurencia dinhii* Masuda *et* Kogame, sp. nov. My Tuong, Phan Rang, Ninh Thuan Province, Vietnam. Figs 19-23. Development of procarps on the terminal segment of female trichoblasts (Figs 19, 20, 22, lateral view; Figs 21, 23, top view); dotted cell in Fig. 23 is an initial cell of the first (lateral) sterile group; ∎ in Fig. 23, supporting cell. Fig. 24. Procarp composed of a four-celled carpogonial branch (dotted) and two sterile groups, lateral (l) and basal (b). Cells of the pericarp on the carpogonial branch were not depicted. a, axial cell; s, supporting cell.

presence of longitudinally oriented secondary pit-connections and a parallel arrangement of its tetrasporangia. Rindi *et al.* (1996) also reported these two features in their specimens of *L. glandulifera* from Calafuria (south of Livorno) in the north-western Mediterranean. The two features are entirely different from those of the alga passing under the name

Figs 25, 26. Flask-shaped cystocarps of *Laurencia dinhii* Masuda *et* Kogame, sp. nov. My Tuong, Phan Rang, Ninh Thuan Province, Vietnam.

L. paniculata (Saito & Womersley, 1974). Further critical studies are clearly needed to elucidate the taxonomic status of these species. *Laurencia concreta* is common along the coast of southern Vietnam and has also been found in Borneo and the Ryukyu Islands, Japan (Masuda, unpublished observations), suggesting that it is probably widely distributed in the tropical to subtropical Pacific.

Laurencia dinhii also produces two periaxial cells from each vegetative axial segment, confirming its subgeneric position within *Chondrophycus* (Nam & Saito, 1995). It is further characterised by the presence of a palisade-like cortical layer and by an attachment system consisting of a single primary discoid holdfast only. Other members of the subgenus *Chondrophycus* with such an outer cortex and ■ attachment system are the ten species: *Laurencia corallopsis* (Montagne) Howe (Yamada, 1931), *L. cruciata* Harvey (Yamada, 1931; Saito & Womersley, 1974), *L. flagellifera* J. Agardh (Yamada, 1931), *L. jejuna* Tseng (1943), *L. longicaulis* Tseng (1943), *L. palisada* Yamada (1931), *L. parvipapillta* Tseng (1943), *L. patentiramea* (Yamada, 1931, as *L. paniculata*), *L. succisa* Cribb (1958, 1983), and *L. tumida* Saito *et* Womersley (1974).

Laurencia corallopsis and *L. patentiramea*, which have broadly ovate (Harvey, 1853, as *L. cervicornis* Harvey) or conical cystocarps (Saito & Womersley, 1974, as *L. paniculata*), respectively, differ from *L. dinhii* with the flask-shaped cystocarps. *Laurencia cruciata* is distinguished from *L. dinhii* by its long intervals between the spreading branches, which arise at wide angles (Yamada, 1931, pl. 5, fig. a; Saito & Womersley, 1974, fig. 24). *Laurencia flagelliformis* (Børgesen, 1937), *L. jejuna* (Tseng, 1943) and *L. longicaulis* (Tseng, 1943) differ from *L. dinhii* by the presence of lenticular thickenings. *Laurencia palisada* differs from *L. dinhii* in its branching, which is distichous in the lower to middle compressed portions and spiral in the upper terete portion (Masuda *et al.*, 1998). *Laurencia parvipapillta* and *L. succisa* are easily separated from *L. dinhii* on the basis of their compressed thalli and distichous branching (Tseng, 1943; Cribb, 1958, 1983). *Laurencia tumida* differs from *L. dinhii* in having thick, rigid, tumid branches, very short, verrucose, tetrasporangial branches, and hemispherical cystocarps (Saito & Womersley, 1974). Thus, *L. dinhii* appears to be well distinguished from all known species of *Laurencia*.

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